Elytra, *Tokyo*, **36**(1): 79–85, May 30, 2008

Molecular Phylogenetic Analysis of the Genus *Actenicerus* (Coleoptera, Elateridae) in Japan

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Abstract The genus *Actenicerus* (Dendrometrinae, Elateridae) is widely distributed in the Holarctic Region and particularly diversified in Japan where approximately 20 species have been recognized. In this study, we examined the relationships of 11 Japanese *Actenicerus*-species based on partial sequences of mitochondrial 16S ribosomal DNA, cytochrome oxidase subunit I "barcode" region, and nuclear 28S ribosomal DNA genes. The result showed that the 11 Japanese *Actenicerus*-species are divided into three major clades.

Introduction

At present, approximately 30 species of the genus *Actenicerus* KIESENWETTER, 1858 (Coleoptera, Elateridae) have been recorded in the Nearctic Region, Europe, Russia, East and Southeast Asia; of these more or less 20 species are found in Japan (ÔHIRA, 1989 a; KISHII, 1996; TARNAWSKI, 2001). Thus, this genus seems to be particularly diversified in Japan. These Japanese *Actenicerus*-species are very similar in external morphology and indeed difficult to distinguish from one another (ÔHIRA, 1989 a). The taxonomic status of some species remains uncertain (ÔHIRA, 1989 a). On the other hand, some Japanese *Actenicerus*-species exhibit distinct niche preferences, such as wetlands or drier land habitats, and some species occur in a limited locality. Hence, Japanese *Actenicerus* is considered to be an interesting group for molecular phylogenetic approach.

Previously, we performed phylogenetic analysis of the Elateridae based on the partial sequences of 28S ribosomal DNA (rDNA) (SAGEGAMI-OBA *et al.*, 2007; OBA,

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2007). The results suggest that *Actenicerus* is placed in the subfamily Dendrometrinae (*sensu* ÔHIRA, 1999; or Prosterinae *sensu* JOHNSON, 2001) and is closely related to the genera *Actenicerомorphus* and *Corymbitodes*. In this study, we analysed phylogenetic interrelationships among 11 Japanese *Actenicerus* taxa based on the partial sequences of mitochondrial 16S rDNA, cytochrome oxidase subunit I (COI), and nuclear 28S rDNA. Additionally, we evaluated the informativeness of "COI barcodes" (HEBERT *et al.*, 2003) for biological identification of Japanese *Actenicerus*-species.

Materials and Methods

The taxa used in this study are listed in Table 1. The DNA extraction method was as described previously (SAGEGAMI-OBA *et al.*, 2007). A partial fragment of 16S rDNA (~600 bp) was amplified by PCR using the primer pair of 16Sar and 16Sbr (SIMON *et al.*, 1994). The PCR condition was 94℃ for 1 min, followed by 40 cycles at 94℃ for 0.5 min, 50℃ for 0.5 min, and 72℃ for 0.5 min, and a final extension step of 5 min at 72℃. The DNA barcode region of COI (~650 bp) was amplified using the primer pair of LCO1490 and HCO2198 (HEBERT *et al.*, 2003). The PCR amplification of partial fragment of 28S rDNA (~880 bp, expansion segments of D1–D3) was performed as previously described (SAGEGAMI-OBA *et al.*, 2007). The amplicons were directly sequenced using BigDye terminator kit 3.1 with ABI PRISM 3130 sequencer (Applied Biosystems, Foster City, CA). All sequences were deposited in GenBank (Table 1). Sequences of each gene were aligned using a L-INS-i strategy of MAFFT 5.734 (KATOH *et al.*, 2005), the regions of ambiguous alignment were manually eliminated, and concatenated together for phylogenetic analysis. Phylogenetic trees were inferred using the maximum likelihood (ML; FELSENSTEIN, 1981), most parsimonious (MP; FARRIS, 1983), neighbor-joining (NJ; SAITOU & NEI, 1987) methods with PAUP*4.0beta 10 (SWOFFORD, 2002), and Bayesian inference with MrBayes 3.1.2 (HUELSENBECK & RONQUIST, 2001). ML and Bayesian inference (1,000,000 generations, burnin=2,500) were performed under a model of GTR+I+Γ (selected by AIC using Modeltest 3.7 and MrModeltest 2.2; POSADA & CRANDALL, 1998). Bootstrap values were calculated as follows: ML, 100 replicates and 10 random taxon addition; MP, 1,000 replicates and 1,000 random taxon addition; NJ, 100,000 replicates under KIMURA's two-parameter (KIMURA, 1980). Based on our previous analysis of the Elateridae (SAGEGAMI-OBA *et al.*, 2007), three outgroup taxa of genera *Actenicerомorphus* and *Corymbitodes* were chosen for rooting the cladogram. The incongruence length difference (ILD) test was performed with PAUP* (FARRIS *et al.*, 1995).

Results

The aligned matrix consisted of 1783 positions (420 for 16S rDNA; 616 for COI; 747 for 28S rDNA), of which 227 positions (56 for 16S rDNA; 159 for COI; 12 for 28S rDNA) were parsimony-informative. Uncorrected pairwise nucleotide differences

Table 1. List of *Actenicerus* and their outgroup taxa used in the analysis.

Species	Collection location	GenBank accession no.			NUM voucher no.
		16S	COI	28S	
Actenicerus aerosus (LEWIS, 1879)	JAPAN: Honshu, Nara, Kasugano, Mt. Kasuga	**AB375485**	**AB375500**	**AB375476**	**NUM Ae02-000086**
Actenicerus athoides (KISHII, 1955)	JAPAN: Honshu, Aomori, Towada, Sarukura	**AB375486**	**AB375501**	**AB375477**	**NUM Ae02-000087**
Actenicerus giganteus KISHII, 1975	JAPAN: Honshu, Mie, Inabe, Fujiwara, Hongo	**AB375487**	**AB375502**	**AB375478**	**NUM Ae02-000088**
Actenicerus kiashianus (MIWA, 1928)	JAPAN: Honshu, Aichi, Seto, Jôkôji	**AB375488**	**AB375503**	**AB231215**	**NUM Ae02-000009**
Actenicerus kidonoi ÔHIRA, 2006	JAPAN: Honshu, Aichi, Okazaki, Kuwabara	**AB375489**	**AB375504**	**AB375479**	**NUM Ae02-000089**
Actenicerus naomii KISHII, 1996	JAPAN: Shikoku, Ehime, Niihama, Douzangoe	**AB375491**	**AB375505**	**AB375480**	**NUM Ae02-000090**
Actenicerus odaisanus (MIWA, 1928)	JAPAN: Honshu, Nara, Kamikitayama, Odaigahara	**AB375492**	**AB375506**	**AB375481**	**NUM Ae02-000091**
Actenicerus orientalis (CANDÈZE, 1889)	JAPAN: Honshu, Shiga, Ika, Yogo, Kushimi	**AB375493**	**AB375507**	**AB375482**	**NUM Ae02-000092**
Actenicerus pruinosus MOTSCHULSKY, 1861	JAPAN: Honshu, Kyoto, Sakyo, Hanase-Sugi	**AB375495**	**AB375509**	**AB375490**	**NUM Ae02-000093**
Actenicerus suzukii suzukii (MIWA, 1928)	JAPAN: Honshu, Aichi, Shinshiro, Naganoyama	**AB375494**	**AB375508**	**AB375483**	**NUM Ae02-000094**
Actenicerus yamashiro KISHII, 1998	JAPAN: Honshu, Kyoto, Sakyo, Hanase-Sugi	**AB375496**	**AB375510**	**AB375475**	**NUM Ae02-000095**
Acteniceromorphus kurofunei (MIWA, 1934)	JAPAN: Honshu, Nagano, Ina, Nishikomagatake	**AB375497**	**AB375511**	**AB231213**	**NUM Ae02-000010**
Corymbitodes gratus (LEWIS, 1894)	JAPAN: Shikoku, Ehime, Saijo, Kawagurusu	**AB375498**	**AB375512**	**AB231231**	**NUM Ae02-000011**
Corymbitodes rubripennis (LEWIS, 1894)	JAPAN: Honshu, Nara, Kamikitayama, Odaigahara	**AB375499**	**AB375513**	**AB375484**	**NUM Ae02-000096**

NUM voucher no., the collection number deposited in Nagoya University Museum.

among 11 *Actenicerus* taxa ranged as follows: 16S rDNA, 0.7–9.0%; COI, 3.2–16.9%; 28S rDNA, 0.0–1.2%. The ILD test for each pair of genes yielded non-significant *P*-values (16S rDNA-COI, $P=0.27$; 16S rDNA-28S rDNA, $P=0.77$; COI-28S rDNA, $P=1.00$), hence the partition homogeneity test supports the combination of the three gene partitions. The χ^2 test of homogeneity of base frequencies across taxa results in no significant *P*-values ($\chi^2=5.0393$, df=39, $P=1.0000$). The optimum ML tree was searched using a heuristic strategy with 100 random sequence addition and TBR branch swapping.

We herein analysed 11 species of Japanese *Actenicerus*, which cover about half of all Japanese taxa (and one-third of the world's taxa) of the genus (KISHII, 1996; TARNAWSKI, 2001). The concatenated sequences of partial 16S rDNA, COI, and 28S rDNA yielded a fully resolved phylogeny. The COI barcode segments were capable of distinguishing among the 11 *Actenicerus*-species: uncorrected pairwise distance between the most closely related species, *A. kidonoi* and *A. giganteus*, was 3.24%; between *A. orientalis* and *A. naomii* was 3.57%; between *A. kiashianus* and *A. yamashiro* was 4.71%.

Discussion

All *Actenicerus*-species analysed in this study form a clade with high statistical supports (100 %, Fig. 1), as previously suggested by morphological studies of larvae; the spiracle of 8th abdominal segment situated at different position (posterior portions of the segment) from the other elaterids (ÔHIRA, 1962, 1989 a). Our results resolved the 11 Japanese *Actenicerus*-species into three distinct clades (Clade A, B, and C; Fig. 1).

Clade A (*Actenicerus pruinosus*, *kiashianus*, *yamashiro*, *suzukii*, and *odaisanus*): Grouping of five *Actenicerus* species in Clade A was supported by ML, MP, NJ, and Bayesian analyses, but the statistical support was moderate (Fig. 1). Within Clade A, three species of *A. pruinosus*, *kiashianus*, and *yamashiro* (named "*pruinosus* group") resemble each other in adult morphology: body slender; antennae elongate and slender especially in male; pronotum narrow, subcylindrical, and the disc bears a median longitudinal groove

Clade B (*Actenicerus orientalis*, *kidonoi, giganteus*, *naomii,* and *athoides*): Grouping of five *Actenicerus* species in Clade B was supported by ML, MP, and Bayesian analyses, but not by MP (Fig. 1). Within Clade B, the monophyly of four species of *A. orientalis*, *kidonoi*, *giganteus*, and *naomii* (named "*orientalis* group") were strongly supported (100 %) by ML, MP, NJ and Bayesian analyses. The "*orientalis* group" share several common characteristics of the adult morphology: body robust; antennae short in both sexes; pronotum subtrapezoidal and the disc does not bear a median longitudinal groove. The phylogenetic tree shows that "*orientalis* group" has diversified relatively recently.

Clade C (*Actenicerus aerosus*): *A. aerosus* occupied the most basal branch in the phylogeny of the 11 *Actenicerus*-species (Fig. 1). The adult morphology is more similar to the species in Clade B than those in Clade A, but some characteristics are distinctly different from all the other members of *Actenicerus* (ÔHIRA, 1989 b); *e.g.*, the outer

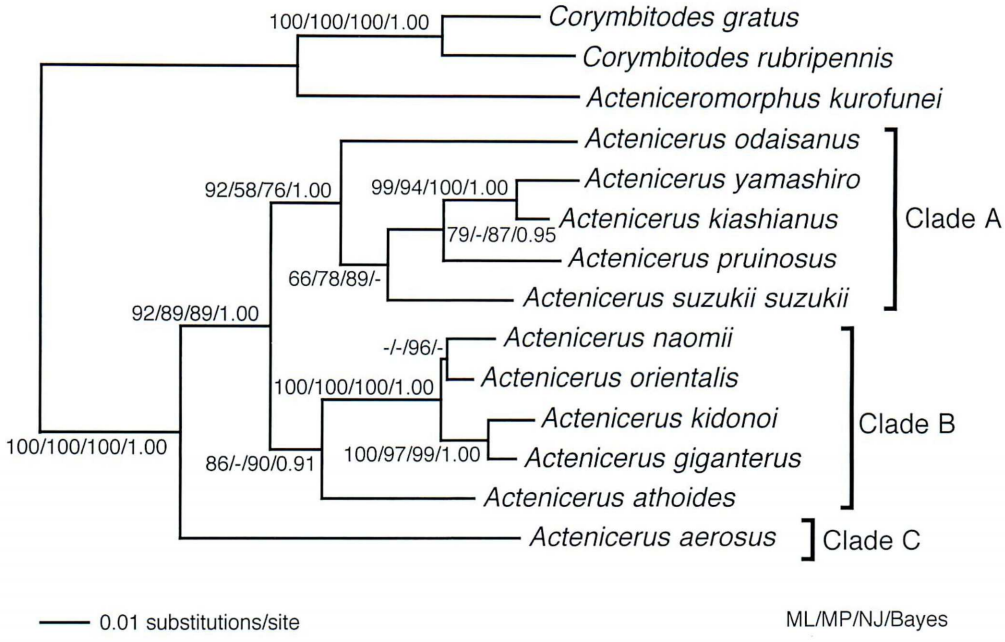

Fig. 1. Phylogenetic tree of 11 Japanese *Actenicerus*-species resulting from ML analysis based on the combination matrix of the partial 16S rDNA, COI, and 28S rDNA ($-\ln L = 4505.4776$). Values of ML/MP/NJ bootstraps/Bayesian posterior probabilities are indicated on the nodes.

prong of urogomphi on 9th abdominal segment is long horn-like upward in the larva of *A. aerosus*, while short horn-like in the other species (ÔHIRA, 1962).

Larvae of *Actenicerus* are generally found in moist soil, but the "*pruinosus* group", *A. pruinosus*, *kiashianus*, and *yamashiro*, are more dry soil inhabiting. Therefore, our molecular phylogenetic results suggest that the ancestral condition in *Actenicerus* was as a wetland inhabitant, and ecological adaptation to dry conditions was derived later in Clade A.

In conclusion, we showed here that the combination analysis of COI with 16S rDNA and 28S rDNA resolved basal relationships of the genus *Actenicerus*, and COI barcode fragment would be potentially useful as a tool for the delimitation of closely related species in this genus. Further analysis, such as comparing various local populations, may help to define the species status of world *Actenicerus*, and hopefully reveal the ecological reasons for explosive diversification of *Actenicerus* in Japan (ÔHIRA, 1989 a, c).

Acknowledgments

The authors thank the following entomologists for materials: Hisayuki ARIMOTO, Hiroshi KIDONO, Toshihiro OZAKI, Masato SHIRAISHI, and Dr. Hiroshi NAKAMURA (Shinshu Univ., Japan).

要　　約

大場(提髪)玲子・大場裕一・大平仁夫：日本産シモフリコメツキ属 *Actenicerus* の分子系統解析．―― 日本から記録されているシモフリコメツキ属 *Actenicerus* は約 20 種にのぼり，きわめて多様なグループを形成している．また，これらの種は外形が類似しているために分類が難しく，系統関係も不明であった．今回，日本産シモフリコメツキ属 11 種について，3 つの遺伝子配列 (16S rDNA, COI, 28S rDNA) に基づく系統推定を行った．その結果，これらの種が大きく 3 つのクレード（クレード A：オオダイルリヒラタコメツキ，キアシシモフリコメツキ，シモフリコメツキ，スズキシモフリコメツキ，ヤマシロシモフリコメツキ／クレード B：オオシモフリコメツキ，クロツヤシモフリコメツキ，サトヤマシモフリコメツキ，シコクシモフリコメツキ，ヨコヅナシモフリコメツキ／クレード C：コガタシモフリコメツキ）に分類されることが初めて示唆された．さらに，解析に用いた COI 領域（DNA バーコード領域）が日本産シモフリコメツキ属の種判別に有効である可能性が示された．

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Elytra, Tokyo, **36**(1): 85–86, May 30, 2008

A Host Record of *Thranius variegatus variegatus* (Coleoptera, Cerambycidae)

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Late in 2007, I recorded *Thranius variegatus variegatus* BATES, 1873 emerged out from dead branches of *Zanthoxylum ailanthoides* SIEBOLD et ZUCC. (Rutaceae) as a distributional record from Kyoto Prefecture, Central Japan (YAMAMOTO, 2007, p. 11). Recentry, I have found out that *Z. ailanthoides* has not been known larval host plant of *T. variegatus variegatus*. In this short paper, I am going to record it as an additional larval host plant of the cerambycid species.

Before going further, I wish to express my hearty thanks to Mr. Satoshi FUJINUMA (Tokyo